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THE PART PLAYED BY LEUCOCYTES IN INFLAMMATION  
IN THE LIGHT OF RECENT BACTERIOLOGICAL INVESTIGATIONS.†

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It is well in the beginning to describe what is meant by the term leucocyte. In the normal blood one finds the following varieties of colorless elements.<sup>1</sup>

(a) Lymphocytes. Small cells about the size of red corpuscles, with a large, round, deeply-staining nucleus, surrounded by a narrow rim of non-granular protoplasm.

(b) Large mono-nuclear leucocytes. These are large cells, several times as large as red corpuscles, with a large oval or elliptical nucleus, and a considerable rim of non-granular protoplasm.

(c) Transition forms Cells with irregular nuclei.

(d) Polynuclear leucocytes. These are about the same size or somewhat smaller than the mono-nuclear variety. The nucleus consists of a long intensely-staining body, which is bent and twisted on itself into bizarre and irregular shapes. Often there seem to be two or three nuclei in the cell. The protoplasm of the cells shows a very fine neutrophilic granulation.

(e) Eosinophiles. Cells of about the same size as the polynuclear form, with a single round, ovoid, or polymorphous nucleus, and containing large, round or ovoid eosinophilic granulations.

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These varieties in the normal blood bear a nearly constant proportion to one another, as follows: lymphocytes, 20 to 30 per cent.; polynuclear, 60 to 75 per cent.; mono-nuclear and transition forms, 6 per cent.; eosinophiles, 2 to 4 per cent.

It has long been known that the only cells that make their way through the vascular walls are the polynuclear leucocytes. These are the principal cells, both as regards numbers and variety, found in the exudations in acute inflammations.

To clearly point out the relations that these cells hold to the inflammatory process and to the other cells, it is necessary to review briefly the part they play.

The first influence of any trauma producing an injury or death of tissue is shown by the various vascular disturbances peculiar to inflammation, and it is not probable that we can have any of the various processes which make up the sum of the vascular disturbances and the wandering out of leucocytes, which we know as inflammation, without a direct lesion, which in most cases takes the shape of a complete necrosis, of the cells of the tissue. The vascular disturbances are shown in a dilatation of the vessels of the part and a retardation of the blood current, and have for their final results the passage of the polynuclear leucocytes through the vascular walls and their accumulation in the surrounding tissue, forming in some cases a definite wall around the point of necrosis, or in other cases forming a diffuse infiltration through the tissue, being accumulated principally in the intercellular spaces. Following this leucocytic invasion there is a proliferation of the fixed cells of the surrounding tissue, leading to the reparation of the injury and the final substitution of cicatricial tissue for the tissue primarily injured and destroyed.

So we may define inflammation as the reaction of living tissues to a trauma. That this is what happens in all inflammations has long been known. But the interesting question of *why* the leucocytes have this property of wandering from the vessels into the tissues, and the exact nature of the conditions and influences under which it takes place, have until recently been a *terra incognita* in pathology. To account for this, many theories have arisen and fallen, and much interesting and valuable work has been done. To review the more recent work that has shed light on these questions is the object of the present paper.

Schlarewsky first and, after him, Weigert, accounted for the accumulation of the leucocytes along the vascular walls, preceding their passage through into the surrounding tissue, in two ways: first, by the physical properties of the corpuscles themselves; and second, by the altered relation of the circulation. That is, the circulation being very much slowed, the corpuscles by their physical properties stick to the sides of the vessel, and thus accumulated they gain their exit by their own active vital movements; or, according to Cohnheim's theory, by a physical process of filtration. But why they make their way from a distance to the point of trauma was left unexplained.

Thus in an inflammation artificially excited in the centre of the cornea the leucocytes make their way from the vessels of the sclera and conjunctiva along the corneal lymph spaces, and finally form a distinct wall around the necrotic point. They show no tendency to pass in the other direction into the sclera and conjunctiva, but pass into the cornea as if irresistibly driven by a *vis a tergo* or drawn thither by a magnet. This process invariably happens at the point of a trauma.

Engelman<sup>2</sup> was the first to appear in this new and rich field of research, which has thrown so much light upon the whole subject of the relations of the leucocytes

and bacteria to inflammation. From his experiments, which showed that many bacteria have an especial attraction for oxygen, and are found in great numbers in the neighborhood of air vesicles accidentally present in the culture fluids, it is learned that these organisms are not to be regarded in the same light as other small but non-vital particles, but that they can be attracted in certain directions.

Before Engelmann, Weigert made the observation that, in blood drawn from patients with relapsing fever and allowed to stand in an open vessel, the spirochaetae of Obermeyer were more numerous on the surface, and advanced, against the law of gravity, from the deeper portions to the surface.

Of more importance than this work is that of various botanists on the higher forms of cells.

Stahl<sup>3</sup> observed that the plasmodium of ethylium septicum, one of the myxomycetæ found in rotten tan bark, can be excited into motion by certain chemical substances contained in the tan bark. If this plasmodium is placed in water on a glass plate, it lies motionless; but if a drop of tan infusion is placed in its neighborhood, the organism moves actively towards this. If, under the same conditions, a drop of a solution of glucose is substituted for the tan infusion, the opposite effect is observed—the plasmodium moving with corresponding rapidity from it. Of especial interest is his observation that the organism can acquire an attraction for certain chemical substances. For instance, if the experiment with the glucose is repeated a number of times, the organism, instead of being repelled, is attracted by it as by the tan infusion.

Du Barry<sup>4</sup>, in his studies on the myxomycetes, has confirmed the work of Stahl. But he further observed that the absorption of solid particles by the plasmodia is determined solely by the chemical properties of the solid matters used. While some organisms will greedily absorb particles of carmine, others will not.

Pfeffer<sup>5</sup> widened our knowledge on these properties of organisms considerably by his researches in 1886. He showed that the spermatozoa of the prothallium of certain ferns were attracted by very dilute solutions of certain chemical substances and that micro-organisms generally were attracted by some substances and repelled by others. His most valuable contribution was, however, to give us a simple and effective method for carrying on such experiments. This method consists in placing very small capillary glass tubes, with one end sealed and partially filled with the chemical substance to be experimented on, in fluids containing the organisms. If the substance has an attraction for the organisms the latter enter the tubes. To this power of attraction he gave the name of chemotaxis. When organisms are attracted to a substance he calls it positive chemotaxis and when they are repelled by a substance negative chemotaxis. In his experiments he found some substances which were indifferent, neither attracting nor repelling organisms.

The first observations made on leucocytes were those of Hess.<sup>6</sup> He sought to learn the fate of pathogenic bacteria injected in pure culture into the circulation of immune animals; that is, animals capable of resisting the organisms so inoculated. For this purpose he injected anthrax bacilli into the circulation of the frog. It had long been known from the results of the observations of Metschnikoff and others that the bacilli are taken up by the leucocytes of the frog, and there undergo gradual destruction. Hess found that, even though immense quantities of anthrax bacilli were injected into the frog's circulation, at the end of three hours very few could be found free in the blood, and at the end of six hours none at all were to be discovered. When Ziegler's plates, between which a small portion of a culture of virulent anthrax bacilli was placed, were introduced

into the tissues of the rabbit, one of the most susceptible animals to anthrax, comparatively few leucocytes entered the space between the plates. If, however, instead of virulent anthrax bacilli, an attenuated form, toward which the animal is immune, was used, the leucocytes entered the space in large numbers, and formed a wall around the organisms.

Lurbarsch<sup>7</sup> found on placing a portion of the lung or spleen of a mouse, dead of anthrax poisoning, into the dorsal lymph space of the frog, that in three days it was enclosed by a wall of leucocytes. He also observed in the immune animal a striking difference between the attraction of the leucocytes for the dead and for the living bacilli.

Lebert,<sup>8</sup> as the result of his studies on the keratitis, produced by the inoculation of the cornea of rabbits with pure cultures of *Aspergillus niger*, came to the conclusion that there is developed at the seat of inoculation a chemical poison, which, becoming diffused into the surrounding vascular tissues, excites inflammatory action. He further found that the injection of dead pus organisms into the cornea would produce an intense purulent inflammation. He succeeded in isolating from the organisms used a chemical substance which would produce inflammation. The substance, to which he gave the name of phlogosin, he supposes exerts an attraction for the leucocytes in the same manner that the substances attracted the plasmodia in Pfeffer's experiments. He found, on placing glass tubes similar to Pfeffer's, partially filled with various substances that excite inflammation, into the anterior chamber of the eye of the rabbit, that they became filled with leucocytes. Other, but indifferent substances, exerted no such attraction. We see from these experiments that the polynuclear leucocytes, which are the only variety capable of amoeboid motion, have the same capacity of being attracted or repelled by certain substances as other amoeboid organisms.

Peckelharing<sup>9</sup> found, on placing small pieces of sponge, some saturated with indifferent fluids and some with anthrax cultures, under the skin of frogs, that the leucocytes enter the latter in much greater numbers than the former.

An extensive series of researches on this property of the leucocytes were carried on after the method of Pfeffer, by Massart and Bordet.<sup>10</sup> They placed tubes filled with various substances in the abdominal cavity and in the tissues of frogs. By a number of substances, prominent among which were cultures of bacteria, leucocytes were attracted. In the tubes containing indifferent substances a few leucocytes were found and their presence was attributed to the tactile excitability of the leucocytes. This, though not so strong as their chemical excitability, was longer preserved.

Gabritchevski<sup>11</sup> in his experiments on the chemotactic power of leucocytes made in Pasteur's laboratory, studied the attraction of leucocytes for bacteria and various other substances. He experimented on frogs, certain lizards, and the subcutaneous tissues of the rabbit, using Pfeffer's tubes. Tubes, containing pure cultures of the various kinds of bacteria experimented with, were allowed to remain in the tissues twenty-four hours. The tubes, on removal, were counted for the number of leucocytes contained. Among other substances the action of jequirity, papayatin, lactic acid, and bicarbonate of sodium was determined. Under similar conditions there were always found more leucocytes in the tubes from the tissues of the rabbit than in those from the frog, sometimes six times as many. He found among the principal substances producing a negative chemotaxis or repellent action on the leucocytes, concentrated solutions of the salts of sodium and potassium, lactic acid in any concentration, quinine, alcohol, jequirity, glycerine and bile; among the indifferent substances, distilled water, phenic acid, phloriozone, glycogen, bouillon and carmine powder suspended in water.

It is an interesting observation that in his experiments fresh cultures of the bacilli of chicken cholera exerted a marked negative chemotactic action on the leucocytes of the rabbit, but with old cultures the result was variable. Of the substances exerting a marked positive chemotactic action, were cultures of various bacteria, especially the pus organisms and the bacillus of typhoid fever. The most striking results were obtained in the rabbit by the typhoid fever bacillus, and in the frog by the bacillus pyocyanus.

Buchner,<sup>12</sup> in a series of articles, has brought out the remarkable fact that it is not the bacteria themselves and the products of their vital activity which have the chemotactic power of attracting leucocytes, but that it is the substance of the bacteria themselves and the chemical constituents of their bodies which attract them. He has succeeded in extracting from the bodies of the bacteria, by subjecting them to the action of a dilute solution of caustic potash, a substance which has a very powerful positive chemotaxis for leucocytes. Such products can also, according to him, be obtained from the necrotic tissues themselves. He finds that the alkali albumins of the products of tissue disintegration exert a similar powerful attraction.

He shows further that certain bacteria produce a local inflammatory action under some circumstances, and a general disturbance with little or no local reaction under others. Thus, the cultures of attenuated anthrax bacilli produce an intense local reaction when inoculated into rabbits, but no general effects are produced; but the cultures of the virulent organisms produce general effects, the animal dying with enormous multiplication of the bacilli in the blood, but with little or no reaction at the point of inoculation.

In man, virulent anthrax bacilli produce primarily a carbuncle, which may or may not be followed by general infection; in the rabbit they are found in large numbers in the blood and the animal dies from septicæmia. In the same manner, the pneumococcus produces a general septicæmia in animals; while in man we find the most active inflammation anywhere known—acute croupous pneumonia. According to Buchner, in all these instances the inflammation, or rather the accumulation of the leucocytes, is produced by the destruction of the bacteria and the liberation of the alkali albumins of their bodies, as well as the alkali albumins produced by the necrosis of the animal tissues.

There is at first glance an apparent difficulty in reconciling these statements with the early lesions found in typhoid fever. This apparent difficulty in the case of the typhoid bacilli, which in the experiments with Pfeffer's tubes are shown to possess a strong positive chemotaxis for leucocytes, while in the early stages of typhoid fever no leucocytes are seen, is cleared up when we consider that in the tissues in the early stages the conditions are so favorable for the bacilli that none of them die, while in the tubes probably many of them perish and then their products attract the leucocytes.

In my own experiments on the so-called organization of the blood-clot, several interesting points in this connection are illustrated. In an ordinary blood-clot in the tissues, to which micro-organisms do not gain access, one of the first changes is the passage into the clot of polynuclear leucocytes. In a large clot, even in a few hours, numbers wander in and scatter in all directions. They are probably attracted into this by the chemical substances resulting from the death of certain elements in the blood. The fibrin present in the clot seems to have an especial attraction for them. When the clot is infected, accidentally or intentionally, a great many more leucocytes wander in. In this case, too, they are scattered diffusely; but, in addition to this, one sees here and there dense areas

of them. These areas are analogous to miliary abscesses. It is an interesting observation that these areas may be found at a distance from the blood supply —far in the clot. This may occur without any suppuration.

The resistance of the blood-clot to infection is an interesting fact of practical importance, as shown by some experiments on the organizing blood-clot in dogs, done by Dr. Welch<sup>13</sup> and myself. We found that the staphylococcus pyogenes aureus does not multiply in the blood-clot; and that, while the number of the leucocytes is greatly increased, the clot infected with these organisms undergoes the same changes as the non-infected clot, and organizes in about the same time. Numbers of leucocytes in scrapings of such an infected clot are seen to contain the micrococci, but the organisms are killed or their virulence is destroyed, probably by the blood-serum in and around the clot, as Nuttal<sup>14</sup> has shown in his blood-serum experiments. The fact that there are more leucocytes seen in the infected than in the sterile blood-clot is probably due to the double attraction of the positive chemotactic power of the normal blood-clot and the bacteria.

What Dr. Welch and I found in the experimentally infected blood-clot accords with the brilliant results of Halsted<sup>15</sup> in the management of the dead spaces of infected wounds with the blood-clot.

That the soluble toxic products of many pathogenic bacteria, especially those of the infectious diseases, exert a positive chemotaxis for leucocytes is an interesting point brought out lately by several observers. Among the more important are the observations of Welch and Flexner<sup>16</sup> in their work on experimental diphtheria. They noticed that in the lesions in the various organs, far removed from the seat of infection, following the necrosis of the tissue-cells, there is an intense infiltration of the necrotic area with leucocytes. In a case of tuberculosis of the knee-joint in which tuberculin was used, the patient afterwards dying of pulmonary tuberculosis, Flexner<sup>17</sup> observed a more extensive necrosis of the tubercles, and much greater numbers of leucocytes than under ordinary circumstances.

Prudden<sup>18</sup> has recently shown in a series of articles that dead tubercle bacilli have a strong positive chemotaxis for leucocytes. In one series of experiments he injected emulsions of sterilized tubercle bacilli into the ear vein of rabbits. At the point of inoculation in a few days there was a raised area containing sterile pus. Wherever the bacilli lodged in the rabbit, there was a dilatation of the surrounding vessels, and an escape of leucocytes into the surrounding tissues.

In a more interesting series of experiments Prudden injected sterilized tubercle bacilli into the trachea of rabbits. In twenty-four hours the cut surface of the lungs showed large numbers of scattered, white, dense, airless areas, corresponding to the bronchi and their adjacent air vesicles. In some places the air vesicles about these areas were congested. The bronchi contained large numbers of tubercle bacilli and leucocytes. Where the number of the bacilli was greatest, the leucocytes were present in proportionately large quantities. In the air vesicles wherever bacilli were present they were accompanied by leucocytes. The mucous membrane of the bronchi was intact. He was not able to isolate a bacterio-protein from the dead tubercle bacilli.

From all these experiments and observations we see that active inflammation at the point of injury, when produced by infection from pathogenic bacteria, is rather a favorable sign than otherwise. For it points out clearly that the tissues, although extensive necrosis may be produced, have the power of destroying and withstanding the effects of numbers of bacteria, and that the leucocytes in the

part and the degree of the inflammatory disturbance are an index of the destruction of the organisms. As we have defined inflammation as the reaction of living tissues to an injury, so we would regard the intensity of this reaction as indicative of the ratio existing between the amount of poison which is to be combatted by the tissues and the amount of resistance of which the tissues are capable.

It is well known, for instance, that those wounds infected at autopsies, at the dissecting table, etc., are the most dangerous in their final results where there is least inflammatory disturbance at the seat of the primary injury. Where there is much local inflammatory disturbance usually the products of the bacteria are resisted and there is no general infection. In the one case there is only the local abscess, with possibly secondary infection of the neighboring lymph glands; and in the other a general septicæmia.

The normal number of leucocytes<sup>19</sup> to the cubic millimetre in the adult is about ten thousand. In some acute diseases, their numbers may rise to thirty-six thousand, but more often it is from fifteen to twenty thousand. In some adynamic conditions the number may sink as low as two thousand.

In this connection Tchistovitch<sup>20</sup> has recently published some interesting researches. He found that after the inoculation of rabbits with attenuated cultures of the pneumococcus, in every case there was an increase in the number of leucocytes in the blood, lasting from one to two days and disappearing on the recovery of the animal. After inoculation of virulent cultures, there was a marked diminution in the number of leucocytes, becoming more evident towards death. In some cases the animal became very ill, and the number of leucocytes greatly decreased; this was followed by a gradual increase in the number of leucocytes, and the animal recovered.

Buchner's theory that it is the chemical substances of the bacterial bodies and the alkali albumins produced by the death of the bacteria that cause the reaction of the tissues, explains many doubtful points in our conception of the mode of infection.

There are many points of practical importance to be derived from this work. Thus, in the treatment of inflammations, we see the worse than useless procedure of trying to abort them by the use of the ice-bag and cold applications, methods that have been and still are largely used in Germany. The very thing that the ice-bag is supposed to do, that is, to lessen the hyperæmia of the part, is the very thing that is most to be avoided. It is desirable to have the most active circulation and hyperæmia of the part affected, both to obtain the effects of the germicidal power of the blood serum in its greatest efficiency in the central point of the inflammation, and to place the surrounding tissues, by the activity of the circulation, in such a condition of nutrition that will best enable them to resist the destructive influences constantly emanating from the central point of infection. It would seem far more rational that—instead of cold—warm applications, such as hot poultices and the hot water bag, should be used. It is not improbable that the greater part of the happy results obtained by the use of the so-called iodoform poultice in acute inflammations is due to the warmth induced. How else can the good results and relief of pain obtained by the use of the actual cautery and massage in acute and chronic inflammations be explained?

As irrational as the use of cold applications, is the use of drugs like ergot and aconite in acute croupous pneumonia. In this disease, too, a similar plan of endeavoring to aid and further the inflammation and not to combat it, should be our guide. In any case it is not the inflammation that is to be feared and

fought—the inflammation being the natural reaction of the tissues to an injury and our friend and ally. But it is the disease, the poison which necessitates the inflammatory reaction, that is to be fought. Unless this is borne in mind and acted upon, those cases that terminate happily will do so in spite of, and not on account of, the treatment received.

Klemperer and Klemperer<sup>21</sup> have recently shown that, in the course of an acute infection with the pneumococcus of acute croupous pneumonia, substances are formed in the blood-serum inimical to the action of the organism, and when a sufficient amount of these substances is formed, crisis takes place. Serum containing these substances, inoculated into another animal suffering with the disease, produces a definite cure. This line of work promises much towards the treatment of infectious diseases in the future.

The whole part that the leucocytes play in the inflamed area we do not yet know. It is certain that they take up living and dead bacteria and the results of tissue necrosis into their bodies and carry them away. Many die in and around the central point of every infection, and it is probable that by their death substances inimical to bacterial products are set free. It is not improbable, also, that by their death substances injurious to bacteria are produced.

In conclusion, I desire to express my indebtedness to Dr. Councilman for many valuable suggestions.

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